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13. ABSTRACT (Maximum 200 words) 1. Serotonin(5HT) Neuron Innervation of the SCN. The 5HT innervation of the monkey SCN was analysed in brains from 5 macaque monkeys using immunocytochemistry with an antiserum against 5HT. There is a dense innervation of the SCN core, the area characterized by receiving direct retinal input. The surrounding area is not innervated but the peribSCN area also has a dense 5HT innervation. In the human brain, this innervation was analysed using an antiserum against the serotonin transporter. Like the monkey, the human SCN has a dense 5HT innervation over the core. These data indicate that the 5HT innervation of the SCN is stable across mammalian species. 2. Clk Gene Expression in the Human SCN. The human clk gene was cloned by Takahashi and his colleagues (Steeves et al, 1999) and an analysis of its localization was performed in this laboratory using in situ hybridization histochemistry. Clk was expressed at high levels in the human SCN, much higher than in adjacent anterior hypothalamus. 3. Clk Gene Expression in the Human Hypothalamus. The expression of clk was low in most hypothalamic areas with the exception of the SCN, paraventricular nucleus(PVH), and the supraoptic(SON) nucleus. In comparing controls against Alzheimer's disease (AD), we found that clk expression was significantly higher in all areas in the AD brains. The interpretation of this finding is unclear. 4. Rhythmicity of clk Gene Expression in the SCN and Other Hypothalamic Areas. The expression of clk was constant in the SCN and other hypothalamic areas in both AD and control brains over the 24 hour period.					
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AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

FINAL TECHNICAL REPORT

'ORGANIZATION OF THE HUMAN CIRCADIAN SYSTEM'

Principal Investigator:

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Project Period: June 1, 1996- November 30, 1998

## TECHNICAL REPORT

**Objectives.** The overall objective of this research program was to provide a detailed analysis of the organization of the human circadian timing system. The objectives for this project period were outlined in the proposal submitted on August 11, 1995. This was subsequently amended in a letter to the Project Officer, Dr. Genevieve Haddad of the AFOSR on August 5, 1997. The specific objectives addressed during the project period were as follows: 1) to determine the pattern of serotonin neuron innervation of the monkey and human suprachiasmatic nucleus (SCN); 2) to determine the pattern of clock (clk) gene expression in the human SCN; 3) to determine the pattern of clk gene expression in the human hypothalamus outside the SCN; 4) to determine whether clk gene expression in the human SCN, and other hypothalamic areas, is rhythmic.

### Research Accomplishments.

1. Serotonin (5HT) Neuron Innervation of the SCN. The 5HT innervation of the monkey SCN was analysed in brains from 5 macaque monkeys using immunocytochemistry with an antiserum against 5HT. There is a dense innervation of the SCN core, the area characterized by receiving direct retinal input. The surrounding area is not innervated but the peri-SCN area also has a dense 5HT innervation. In the human brain, this innervation was analysed using an antiserum against the serotonin transporter. Like the monkey, the human SCN has a dense 5HT innervation over the core. These data indicate that the 5HT innervation of the SCN is stable across mammalian species.
2. Clk Gene Expression in the Human SCN. The human clk gene was cloned by Takahashi and his colleagues (Steeves et al, 1999) and an analysis of its localization was performed in this laboratory using *in situ* hybridization histochemistry. Clk was expressed at high levels in the human SCN, much higher than in adjacent anterior hypothalamus.
3. Clk gene Expression in the Human Hypothalamus. The expression of clk was low in most hypothalamic areas with the exception of the SCN, paraventricular nucleus (PVH), and the supraoptic (SON) nucleus. In comparing controls against Alzheimer's disease (AD), we found that clk expression was significantly higher in all areas in the AD brains. The interpretation of this finding is unclear.

Diagnosis	Mean Optical Density		
	SCN	PVH	SON
Control	0.13±0.03	0.13±0.02	0.09±0.01
Alzheimer's Disease	0.21±0.08	0.28±0.03	0.34±0.08

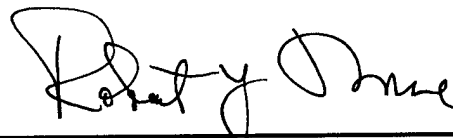
4. Rhythmicity of clk Gene Expression in the SCN and Other Hypothalamic Areas.  
The expression of clk was constant in the SCN and other hypothalamic areas in both Ad and control brains over the 24-hour period.

Publications.

Steeves, TDL, King DP, Zhao,Y, Sangoram, AM, Du, F, Bowcock, AM, Moore RY and Takahashi, J (1999) Molecular cloning and characterization of the human CLOCK gene: Expression in the suprachiasmatic nuclei. Genomics 57: 189-200.

Moore, RY and Speh, JC (2001) Serotonin innervation of the monkey and human suprachiasmatic nucleus. Brain Research, submitted.

Inventions, Patents. None



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Robert Y. Moore, M.D.,Ph.D.  
Principal Investigator